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BEHAVIORAL EFFECTS OF COMBINED PERINATAL L-NAME AND 0.5 Hz MAGNETIC FIELD TREATMENTS

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The behavioral effects of the nitric oxide synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME), when perinatally (2 d prenatal–14 d postnatal) co-administered with extremely low frequency magnetic fields, were examined in weanling and adult rats. Litters of rat pups and their dams were exposed continuously to biphasic pulsed fields presented once every 2 s. The magnetic fields were amplitude modulated in successively increasing and decreasing steps (each 30 min) between 0 and 1.8 microT or between 0 to 13 nanoT (reference field) during 4-h periods (6 periods per day). These two treatments were subdivided into dams that received tap water and dams that received 1.0 g/L L-NAME in tap water. The behavioral sequelae to these treatments for 242 progeny from 41 litters were followed from weaning (1 wk after termination of treatment) into adulthood. Compared to exposures to water and nanoT magnetic fields, perinatal exposures to the microT magnetic fields or to L-NAME in the maternal water supply were associated with increased activity levels when the rats were tested as weanlings, but decreased activity levels when the rats were tested as adults. However, the activity of rats that received the combination of L-NAME and microT magnetic

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fields did not differ significantly from the activity of the rats that had received water and the nanoT fields. Long-term (adulthood) effects of these perinatal treatments on associative learning, as inferred by learned fear to contextual stimuli, were not evident. These results indicate that L-NAME and this particular pattern of magnetic field antagonized one another when co-administered during the perinatal period.

Keywords behavior, ELF, magnetic fields, nitric oxide, perinatal, rat

Nitric oxide (NO), a labile free radical gas, is synthesized from the amino acid L-arginine by nitric oxide synthase or NOS (Snyder & Dawson, 1995) and acts as a diffusible retrograde modulator of synaptic plasticity in the adult nervous system (Kandel, 2000). Nitric oxide may be implicated in developmental processes within the nervous system (Kandel & O'Dell, 1992). Chronic postnatal inhibition of NOS by N-nitro-L-arginine methyl ester (L-NAME) markedly reduced basal cGMP levels (Prickaerts, de Vente, Markerink-van Ittersum, & Steinbusch, 1998) and NADPH diaphorase activity (Virgili, Monti, LoRusso, Bentivogli, & Contestabile, 1999) in the neonatal rodent. These levels returned to normal within a few days after termination of treatment (Prickaerts et al., 1998; Virgili et al., 1999), suggesting that profound depressions in the inferences of nitric oxide efficacy during the postnatal period were reversible. The behavior of these rodents as adults was indistinguishable from untreated controls (Wortwein, Gustafson, Liv Hansen, & Mogensen, 1997; Prickaerts et al., 1998).

Cumulative evidence indicates that extremely low frequency (ELF; <100 Hz) magnetic fields modulate nitric oxide activity. The first demonstration of this effect was reported by Bawin, Satmary, Jones, Adey, and Zimmerman (1996) who noted that 1 Hz magnetic fields could destabilize rhythmic slow activity in hippocampal slices. This response was abolished when NO was pharmacologically inhibited. Subsequent work by Kavaliers, Choleris, Prato, and Ossenkopp (1998) convincingly demonstrated that magnetic fields modulated nitric oxide activity. These authors reported that the inhibition of enkephalin-mediated antinociception by 60 Hz magnetic fields (Tysdale, Lipa, Ossenkopp, & Kavaliers, 1991) could be significantly enhanced by the NO releasing agent S-nitro-N-acetylpenicillamide (SNP) and significantly reduced by the NOS inhibitor L-NAME. Other authors

have reported modulation of NOS activity by magnetic fields. When the dependent measure was NO spin-trapping followed by electron paramagnetic measurement, it was noted that only ELF fields co-administered with lipopolysaccharides augmented NOS generation (Yoshikawa et al., 2000). When the dependent measure was NOS activity in the cerebellum only pulsed DC fields, but not ELF AC fields, augmented NOS activity (Noda, Mori, Liburdy, & Packer, 2000).

We (McKay, St-Pierre, & Persinger, 2001; Persinger, St-Pierre, & Koren, 2001) have found that rats exposed prenatally to complex (temporally asymmetric and physiologically relevant) magnetic fields exhibited marked hyperactivity as weanlings and impoverished associative memory as adults. The specific deficits reported in these studies were always specific to the shape of the applied waveform. The pharmacological bases to these effects were not pursued although the findings were consistent with the modification of acute pharmacological nitric oxide.

In the present study, we examined the interaction between perinatal (2 d prenatal through 14 d postnatal) L-NAME (1.0 g/L administered through the drinking water of the dams) and continuously applied ELF magnetic fields (0.5 Hz amplitude modulated within the nanoTesla range or the microTesla range over 4-h periods, 6 times per day). The magnetic field characteristics were designed from the results of correlational studies (O'Connor & Persinger, 1999) that had indicated pcl (continuous pulsation; 0.2–5 Hz) geomagnetic micropulsations with intensities of less than 10 nT were associated with increased monthly incidences of sudden unexpected deaths in human infants. The dose of the NOS inhibitor L-NAME was selected on the basis of results from a pilot study (O'Connor & Persinger, unpublished observations).

We measured open field behavior in weanlings because previous research with prenatal magnetic field exposures (Persinger, 1969; Ossenkopp, 1972; Ossenkopp & Ossenkopp, 1983; Persinger, St-Pierre, & Koren, 2001) and the effects of chronic nitric oxide inhibition on pup ambulation (Prickaerts et al., 1998) had cumulatively suggested the sensitivity of this measure. During adulthood we explored the consequences of these perinatal treatments upon associative learning (contextual fear conditioning paradigm) and latencies

of nociceptive responses to thermal stimuli. These behavioral endpoints were chosen because the former is unequivocally refractory to nitric oxide modulation (Maren, 1998; Johnson, Baker, & Azorlosa, 2000), yet is vulnerable to specific shapes of applied complex magnetic fields (McKay, Persinger, & Koren, 2000). The latter may be modulated by both applied magnetic fields and nitric oxide donors/NOS inhibitors (Kavaliers et al., 1998; Dixon & Persinger, 2001).

MATERIALS AND METHODS

Subjects

A total of 48 female and 24 adult male Wistar rats (Charles River, Quebec) were employed as breeders. Rats were maintained 3/cage in standard colony conditions from the time of their arrival into the colony (at approximately 60 d of age) until approximately 90-120 d. Food and water were always available ad libitum. Photophase onset was 0730 h local time with 12:12 L:D cycle. Temperature was maintained at 20 ± 1°C. The study was completed in 4 blocks of 12 females per block over approximately 4 months.

Perinatal Treatment

Breeding

Male rats were housed singly for approximately one week, after which time nulliparous female rats were introduced singly into the males' home cages. A trained observer monitored the presence of sperm plugs daily, shortly after the onset of the photophase. Sperm plug dates for each breeding pair were used to estimate parturition dates. Females remained housed with the males for approximately 2 wk after which they were single housed in 35 cm × 30 cm × 18 cm plastic cages with one-quarter inch corn cob bedding. Female rats were placed into the magnetic field exposure room 2 d prior to the expected parturition date. Each male was employed twice at approximately 30-d intervals to breed the nulliparous females.

Magnetic Field and L-NAME Treatments

Once in the exposure room, the females' cages were placed into the custom constructed coils. Detailed descriptions of these coils have been published elsewhere (Stewart & Persinger, 1999). Briefly, each coil (one oriented in the N-S direction and one in the E-W direction) consisted of a large (112 cm × 125 cm) aluminum rack wrapped around its perimeter by 72 turns of 30 gauge (AWG) copper wire (total 13 cm wide). The females' cages (standard plastic cages with aluminum tops containing food and the water bottle) were placed on shelves within the racks. According to a MEDA FM-300 magnetometer, the strengths of the resultant steady magnetic field, primarily from the earth, ranged between 43,055 nT to 47,500 nT in one coil and 43,218 nT to 54,747 nT in other coil.

During any given block, only one coil was connected to custom-constructed circuits designed to generate a pair of opposite polarity pulses (separated by 0.5 s) that were presented every 2 s. The circuit had initially been constructed to generate a square wave. However, when coupled to the coil, positive and negative pulses were generated during the rise and the fall of the square wave (0.5 s plateau). Because the time chip exhibited a rise/fall time of 100 to 300 nanos and the amplifier that drove the output had a rise time of 0.3 to 0.7 V/micros, this value at 5 V was between 1.5 and 3.5 micros.

The 0.5 Hz "square-pulse" was amplitude-modulated during successive 4-h cycles with 6 cycles per day. Within each cycle the intensity of the field increased and decreased in four separate steps each lasting for 30 min. The maximum intensities of the amplitude modulations according to a magnetometer during each step were 0, 870 nT, 1040 nT, 1300 nT, and 1770 nT. Within the portion of the cage furthest from the coil these values were 0, 790 nT, 950 nT, 1200 nT, and 1650 nT. The amplitudes then decreased in the reversed order. An example of a magnetogram for a single day is shown in Figure 1.

When the active coil was emitting its field, a weaker field, ranging from 0 to 4 nT, 8 nT, 11 nT, and 13 nT before reversing the order of intensity, was measured in the reference coil that was not connected to any equipment (open-circuit). The lowest intensity within the boundaries of the cages for these sequences were 0, 1 nT, 3 nT,

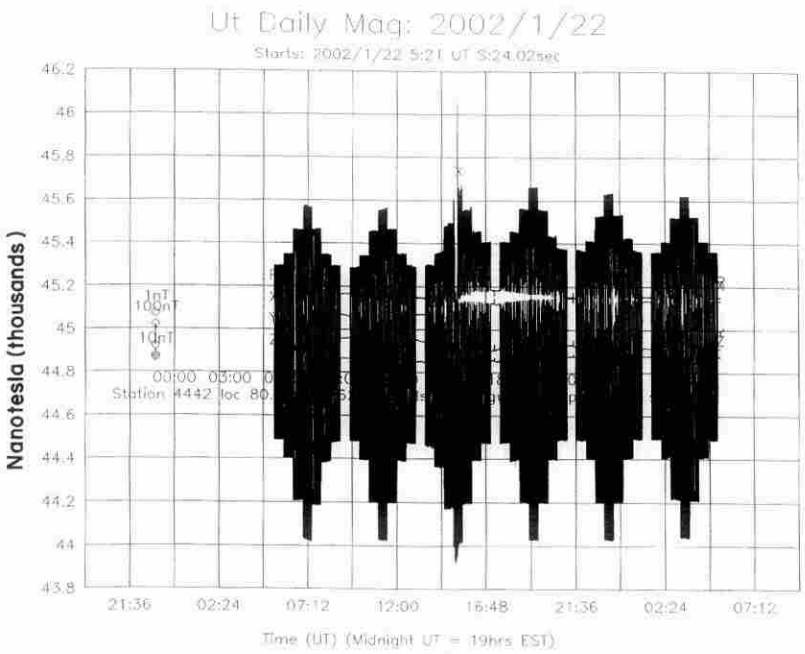

FIGURE 1. Sample pattern of the experimentally produced amplitude modulations of a 0.5 Hz magnetic field (microT range) produced within an activated coil during one 24-h period. The normal variations of the geomagnetic field are masked.

6 nT, and 8 nT. These experimentally induced amplitude modulations were often superimposed upon the normal variations in geomagnetic activity. An example of this superimposition during mildly unsettled geomagnetic conditions is shown in Figure 2.

During each block of the experiment, six cages of females were placed into the activated coil and six were placed into the reference coil. The activated coil and the reference coil were alternated between successive blocks of the experiment by disconnecting one coil and plugging the field generator into the other coil. The spatial location of each coil remained constant across experimental blocks. Within each coil one-half the numbers of the females received tap water to drink and the other half were given L-NAME (Sigma) in tap water (1.0 g/L). These treatments were initiated 2 d prior to the expected parturition date and continued until 14 d after parturition.

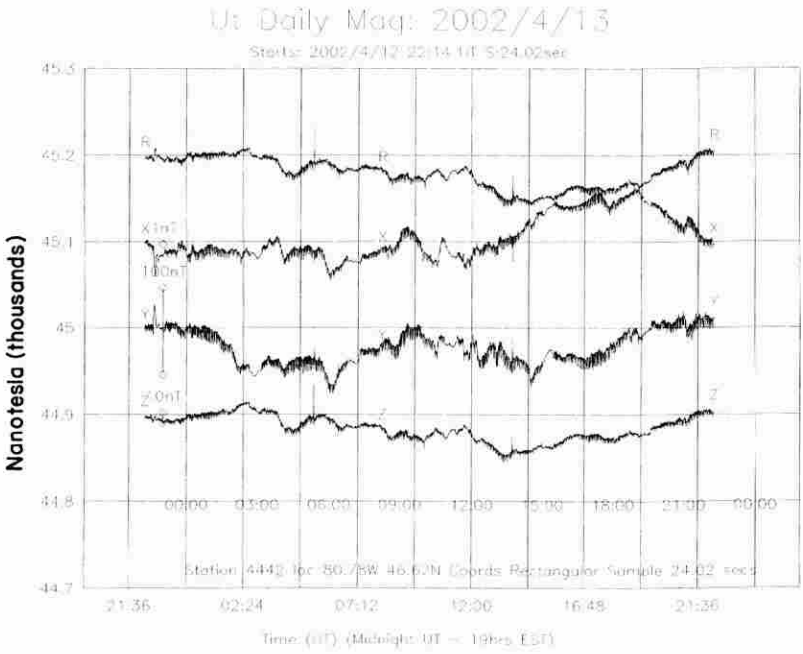

FIGURE 2. Sample pattern of the magnetic field induced within the reference coil by the activated coil during a sample 24-h period. The successive steps in the amplitude changes in the 0.5 Hz variations are superimposed upon the geomagnetic activity. The shapes for the X, Y, Z components, as well as the resultant fields, are presented for comparisons. The absolute intensity of the field (vertical axis) is for discerning the amplitude variations, not the steady-state value of the specific components.

The mothers and pups were relocated to another room at this time; pups were weaned at 22 d of age.

Open Field Behaviors

Open field testing was conducted at 22 d of age for 3 male and 3 female pups randomly selected from each litter (total litters = 41; total pups = 242). Because specific combinations of the perinatal treatments were associated with significant mortality (Persinger & McKay, unpublished observations), random selection of 3 pups of each sex from each litter was not always possible. Pups were placed singly for 2 min into a 60 cm × 60 cm × 30 cm custom constructed open field box (Persinger, 1969); a grid of 12 cm squares had been

traced on the floor of the open field box beneath a superficial layer of varnish. The open field arena was cleaned with a dilute solution (0.4%) of aqueous acetic acid, and then dried, prior to testing each pup. During the observation period the numbers of squares traversed (defined as the movement of the entire body from one square into an adjacent square), rearings (defined as the assumption of an upright stance on the two hind limbs), grooming episodes, fecal boluses, and urinations were recorded for each pup. Upon removal from the open field arena pups were weighed, ear punched for identification, and relocated 3/cage (same sex) to standard colony housing conditions. The rats were weighed at 30 d intervals until they were 110 d old.

Contextual Conditioned Fear

At approximately 90 d of age, rats from the first two blocks of the experiment (N = 133) were fear conditioned to contextual stimuli. The procedure has been described in detail elsewhere (Stewart & McKay, 2000). Briefly, rats were placed into a modified operant (contextual) chamber connected to an A-615-C Master Shocker (Lafayette Instruments) programmed to deliver scrambled footshocks. On the conditioning (training) day, rats were placed into the contextual chamber and given a 3-min habituation period, during which time the numbers of midline chamber crossovers, defined as forward movements of the rat across the midline of the chamber (an inference of pre-shock baseline motoric activity), were recorded. The habituation period was followed by the delivery of 3 unsignaled 2-s, 0.5 mA footshocks at 60-s intervals.

Freezing, defined as immobility except that strictly necessitated by respiration, was recorded nominally (0 = movement, 1 = freezing) every 8 s during each 60-s post-shock interval (total of 7 observations per post-shock period). After the last 60-s observation period rats were immediately returned to their home cages. Contextual retention testing was conducted 24 h later. Rats were returned to the contextual chamber in which conditioning sessions had taken place and for 8 min were scored for defensive freezing (same procedure as described above). This resulted in a total of 60 observations. The percent duration of freezing during retention testing was calculated

as the numbers of freezing observations divided by the total numbers of observations. The chamber was cleaned with a dilute solution (0.4%) of aqueous acetic acid and then dried prior to conditioning or testing each rat.

Hotplate Analgesia

At approximately 100 d of age rats (N = 241) were assayed for latency to respond to a thermal stimulus in a Hotplate Analgesiometer (Omnitech). Each rat was placed into the hotplate apparatus (55°C). Each trial ended when the rat displayed two hind paw licks (each hind paw once, or one hind paw twice) or when 60 s had elapsed.

Statistical Analyses

Five-way analyses of variance (ANOVA; drug: water versus L-NAME; field: reference versus synthetic field; sex: male versus female; coil: N-S versus E-W orientations; coil order: first versus second activation of each coil), and six-way multivariate analyses of variance (MANOVA; identical to the ANOVAs, except four age intervals, were included as repeated measures) were employed to discern the effects of these independent levels on the dependent measures listed above. Fear conditioning data were assessed by four-way ANOVA (the independent level for order of coil activation was removed as only the rats derived from the first half of the study were tested). Criterion for statistical significance was set at $p < .02$. Because the effects of the perinatal drug treatments and perinatal magnetic field treatments (and their possible outcomes as a function of sex) were of primary interest to the study, other levels in the analyses were included for the purpose of covariance.

The algorithm employed by ANOVA, which extracts residual variance from all independent variables before discerning the level of statistical significance for main effects and interactions between other variables entered into the analyses, enables one to reliably report main effects and lower order interactions as statistically significant, despite higher order interactions which may also be statistically significant. For the present study, higher order interactions that were statistically significant explained trivial (<1–2% explained)

variance and are not reported. Due to the scope of the multi-way analyses undertaken, all main effects and interactions (all dfs = 1,210, except fear conditioning where dfs = 1,117) that did not reach criterion for statistical significance are also not reported.

We had reasoned that the combination of L-NAME treatments and synthetic magnetic field treatments might cancel the effects of each of these treatments when applied singly. This result would be supported by statistically significant two-way interactions between perinatal drug and field treatments. To discern the source of the interaction we employed Student Neuman-Keuls (SNK) tests as post hoc analyses. To examine the trend in the values of the relevant dependent measures, we employed polynomial analyses with the groups placed in the following order: water/nanoT field, water/microT 0.5 Hz field, and L-NAME/nanoT field, L-NAME/microT 0.5 Hz field. Effect sizes reported are multiple R^2 from the ANOVAs (equivalent to the squared correlation coefficient derived from regression analyses) and partial eta^2 from the MANOVAs. All analyses were completed with SPSS software on a VAX 4000 computer.

RESULTS

Body Weight

The results of multivariate analysis of variance (MANOVA) showed robust differences in body weights between male and female rats [$F(1,209) = 1769.35$, $p < .001$, $eta^2 = 89\%$] and a robust interaction between sex and age interval [$F(3,627) = 2117.92$, $p < .001$, $eta^2 = 91\%$]. Because of these marked differences in body weight between the sexes, we reasoned that separate MANOVAs for each sex were warranted. Table 1 reports the body weights of male and female rats at each age interval for L-NAME versus tap water treated rats, and field-exposed versus reference-exposed rats.

Males

MANOVA demonstrated a statistically significant difference in body weights between rats that had received the perinatal tap water and

TABLE 1. Means and standard errors of the means (SEMs) for male and female rats' body weights at 22, 50, 80, and 110 d for rats given A: tap water or 1.0 g/L of L-NAME perinatally, and B: microTesla or nanoTesla 0.5 Hz magnetic fields perinatally

		22 d		*50 d*		*80 d*		*110 d*	
	N	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
A									
Male									
Water	68	42.4[a]	1.0	269.1[a]	3.9	434.0[a]	4.5	497.7[a]	5.8
L-NAME	52	37.3[b]	0.7	255.7[b]	3.5	417.1[b]	6.3	473.8[b]	6.4
Female									
Water	68	39.3[a]	0.9	186.3	2.2	258.3[a]	2.7	281.1[a]	3.2
L-NAME	54	36.3[b]	0.8	180.2	2.2	245.8[b]	3.7	270.8[b]	3.1
B									
Male									
nanoT	57	41.3	0.9	267.1	3.7	433.4	5.0	494.9	6.2
microT	63	39.2	1.0	260.0	4.0	420.1	5.6	480.7	6.2
Female									
nanoT	60	38.5	0.9	187.5[a]	2.2	258.9[a]	2.6	282.6[a]	3.0
microT	62	37.5	0.9	180.0[b]	2.2	246.9[b]	3.6	270.1[b]	3.3

[a]versus [b]post hoc $p < .05$.

those that had received the perinatal L-NAME administration [$F(1,103 = 9.69$, $p < .01$, eta$^2 = 9\%$]. Differences in weights between rats that had been exposed to magnetic field or reference conditions were not significant statistically [$F(1,103] = .70$]. A marked increase was noted in weights across age intervals [$F(3,309) = 12269.29$, $p < .001$, eta$^2 = 99\%$]. A statistically significant interaction between age interval and perinatal drug treatment groups was apparent [$F(3,309) = 4.79$, $p < .01$, eta$^2 = 4\%$]. However, an interaction between age interval and perinatal magnetic field treatment groups was not evident [$F(3,309) = .99$, n.s.]. One-way analyses of variance indicated that the magnitude of weight gain between age intervals for male rats did not differ significantly between perinatal drug and perinatal field treatment groups [$Fs(1,117) < 3.31$, n.s.].

Females

MANOVA showed statistically significant differences in body weights between perinatal tap water and perinatal L-NAME treated rats [$F(1,106)$

= 7.40, $p < .01$, eta^2 = 7%] and between rats exposed perinatally to reference conditions and those exposed to the magnetic fields [$F(1,106) = 7.35$, $p < .01$, eta^2 = 7%]. Robust increases in body weights were apparent across the four measurement intervals [$F(3,318) = 10786.51$, $p < .001$, eta^2 = 99%]. Statistically significant interactions were noted between age intervals and perinatal drug treatments [$F(3,318) = 3.66$, $p = .01$, eta^2 = 3%] and between age interval and perinatal magnetic field treatment groups [$F(3,318) = 5.57$, $p = .001$, eta^2 = 5%]. One-way analyses of variance indicated that female rats given L-NAME gained less weight between 50 d through 80 d of age compared to the rats that received only tap water [$F(1,120) = 4.75$, $p < .05$]. The female rats that had been exposed to the microT magnetic field (activated coils) weighed less between weaning and 50 d of age compared to those female rats exposed perinatally to the nanoT (reference) fields [$F(1,120) = 6.84$, $p = .01$]. No other differences in weight gain between age intervals for field-treated or drug-treated groups were noted for female rats [$Fs(1,120) < 2.23$, n.s.].

Open Field Behaviors

Square Traversals

Five-way ANOVA showed a statistically significant difference in total numbers of squares traversed during open field sessions between male ($M = 37.14$, $SD = 22.41$) and female ($M = 46.88$, $SD = 23.07$) rats [$F(1,210) = 14.75$, $p < .001$, multiple R^2 = 4%], and between rats given perinatal tap water ($M = 44.67$, SD = 22.01) and perinatal L-NAME ($M = 38.69$, $SD = 24.37$) [$F(1,210) = 6.62$, $p = .01$, multiple R^2 = 2%]. A statistically significant interaction between perinatal drug treatments and perinatal magnetic field treatments was noted [$F(1,210) = 21.36$, $p < .001$], due to the increased numbers of squares traversed by rats that had been given tap water and exposed to microT magnetic fields, relative to those given tap water and exposed to the reference (nonactivated) coil or those given L-NAME and exposed to the microT magnetic fields. Rats that received L-NAME perinatally and had been exposed to the nanoT fields were significantly less active than those rats that re-

ceived perinatal L-NAME and received the microT magnetic field treatments. The quadratic term for this interaction was also statistically significant [$F(1,238) = 20.21$, $p < .001$].

Rearing

Five-way ANOVA showed a statistically significant two-way interaction between perinatal drug treatments and perinatal magnetic: field applications [$F(1,210) = 11.48$, $p = .001$]. The source of this interaction was the significantly greater numbers of rearings during open field testing displayed by the rats that had received tap water and exposed to the microT magnetic field, relative to those rats given tap water and exposed to the nanoT fields or the rats given perinatal L-NAME and exposed to the microT magnetic fields. The latter two groups did not differ significantly from each other (Table 2). The quadratic term was statistically significant [$F(1,238) = 10.04$, $p < .01$].

Grooming

Five-way ANOVA extracted a statistically significant two-way interaction between perinatal drug and perinatal magnetic field treatment groups [$F(1,210) = 10.83$, $p = .001$]. Post hoc analyses indicated that rats that had been given tap water and had been exposed to the microT magnetic fields groomed significantly more often during open field testing than did rats given tap water and exposed to the nanoT fields. The quadratic for this interaction was also statistically significant [$F(1,238) = 7.42$, $p < .01$].

Defecation

Five-way ANOVA discerned a statistically significant difference in defecation during open field testing between rats given perinatal tap water ($M = 0.51$, SEM = 0.07) and rats given perinatal L-NAME ($M = 0.83$, SEM = 0.09) [$F(1,210) = 9.38$, $p < .01$, multiple $R^2 = 4\%$]. A statistically significant interaction between perinatal drug treatments and sex was also apparent [$F(1,210) = 5.98$, $p < .02$]. Post hoc analyses discerned the source of the interaction to be the

significantly greater frequency of defecation for male rats given perinatal L-NAME (M = 1.08, SEM = 0.12), relative to male rats given perinatal tap water (M = 0.50, SEM = 0.10), the female rats given perinatal tap water (M = 0.53, SEM = 0.09), or the female rats given perinatal L-NAME (M = 0.59, SEM = 0.12). The last three groups did not differ significantly from each other.

Urination

There were no remarkable findings from the five-way ANOVA for urination during open field testing.

Contextual Conditioned Fear

Midline Crossovers

Four-way analysis of variance revealed statistically significant differences in the numbers of midline chamber crossovers prior to context/shock pairings between male (M = 7.95, SD = 2.60) and female rats (M = 10.40, SD = 2.60) [$F(1,117) = 30.65$, $p < .001$, multiple R^2 = 18%]. A statistically significant interaction between perinatal drug administration and perinatal magnetic field treatment [$F(1,117) = 7.41$, $p < .01$] was also found. Post hoc analysis did not unambiguously discern the source of this latter effect, although the quadratic term of the polynomial analysis was significant statistically [$F(1,129) = 6.08$, $p < .02$].

Post-Context/Shock Freezing

There were no remarkable findings from the four-way ANOVA for the duration of freezing following the delivery of footshocks.

Freezing During Retention Testing

Four-way ANOVA discerned a statistically significant difference [$F(1,117) = 27.11$, $p < .001$; 17% of variance explained] in the durations of freezing behavior during contextual retention testing between male rats (M = 79.9%, SD = 17.8) and female rats (M =

62.6%, *SD* = 20.1). Because differences between the treatment groups in baseline motor activity existed prior to conditioning, an additional ANOVA was completed that first removed the variance attributed to baseline motor activity. Differences in freezing between the sexes remained statistically significant. No other differences were statistically significant.

Hotplate Analgesia

Five-way ANOVA discerned statistically significant differences in hotplate latencies between male (M = 15.33, SD = 6.68) and female (18.14, SD = 7.54) rats [$F(1,210) = 14.74$, $p < .001$, multiple R^2 = 4%]. A statistically significant two-way interaction was noted between perinatal drug treatments and perinatal magnetic field treatments [$F(1,210) = 5.87$, $p < .02$]. Although the source of the interaction between perinatal drug and magnetic field treatments was ambiguous by SNK post hoc analysis, inspection of the means and SEMs for the four combinations of drug and field treatment groups suggested the likely source of the interaction was the reduced hotplate latencies for rats given perinatal L-NAME and exposed to the nanoT magnetic fields, relative to those rats that received both L-NAME and the microT magnetic fields. Significant overlaps of the SEMs for rats given perinatal tap water and exposed to either nanoT or microT magnetic field conditions were evident (Table 2).

DISCUSSION

This study examined the behavioral effects of the nitric oxide synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME) when co-administered with extremely low frequency (0.5 Hz) magnetic fields during an extended perinatal period. Seven days following termination of these treatments, pups displayed increased activity levels relative to reference groups. As adults, these rats were hypoactive. Application of the microT magnetic fields antagonized the effects of L-NAME when the two agents were co-administered. Long-term (adulthood) effects of these perinatal treatments on associative learning, as inferred by learned fear to contextual stimuli, were not evident

TABLE 2. Means and standard errors of the means (SEMs) for numbers of square traversals, rearings, and defecations during open field testing after weaning, the numbers of midline chamber crossovers prior to context/shock pairing, and the latencies to responding to the hotplate (s) as adults for rats given tap water or 1.0 g/L of L-NAME and either nanoT or microT 0.5 Hz magnetic fields perinatally

	*Squares**		*Rearing**		*Grooming**	
Group	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
Water/nanoT field	37.89[a]	2.49	8.76[a]	0.58	1.26[a]	0.12
Water/microT field	51.86[b]	2.59	11.26[b]	0.68	1.71[b]	0.14
L-NAME/nanoT field	44.70[c]	4.06	10.30	0.77	1.70	0.14
L-NAME/microT field	33.90[a]	2.63	8.93[a]	0.53	1.46	0.11
	*Midline**		*Hotplate***			
	Mean	*SEM*	*Mean*	*SEM*		
Water/nanoT field	10.06	0.53	17.00	0.93		
Water/microT field	8.56	0.43	16.62	0.85		
L-NAME/nanoT field	8.52	0.46	14.93	0.71		
L-NAME/microT field	9.42	0.51	18.09	1.11		

[a]versus [b]and [a]versus [c]post hoc $p < .02$.
Quadratic term for group order as listed: *$p < .05$, **$p = .06$.

although minor changes in motor activity and response latencies to thermal stimuli were noted.

Voelker et al. (1995) have reported that perinatal administration of L-NAME, either intraperitoneally or through the mother's breast milk, resulted in a marked retardation of growth without evidence of compensatory growth into adulthood. At 14 d postnatal, the endpoint of these authors' study, it was noted that the ratio of stomach weight to pup weight was significantly increased in L-NAME-treated rats relative to controls (indicative of L-NAME-induced hypertrophic pyloric stenosis). Prickaerts et al. (1998) reported similar findings. This deficit in growth and absence of compensatory growth as inferred by changes in growth rate attributed to nutritional sources by Voelker et al. (1995) may have contributed to the lower body weights of L-NAME-treated rats noted in the present study.

In a separate study (Persinger & McKay, unpublished observations), we noted that stomach weight to pup weight ratios at 21 d of age were markedly reduced in L-NAME-treated pups, a finding

contrary to that reported by both Voelker et al. (1995) and Prickaerts et al. (1998). As our study assayed stomach weights 7 d following the termination of treatment, compared to assaying stomach weights on the last day of treatment, this marked difference in results may suggest a rebound change in nitric oxide regulation of the pyloric sphincter. This response would be similar to rebound changes in nitric oxide-related enzymatic activities for cGMP levels reported by Prickaerts et al. (1998) and for NADPH diaphorase activity reported by Virgili et al. (1999). Our finding of increased incidences of defecation during open field testing for L-NAME-treated rats relative to untreated rats further supports a rebound in nitric oxide regulation of the pyloric sphincter.

Acute treatments with nitric oxide synthase inhibitors have been shown to reduce baseline motor activity in rodents (Sandi, Venero, & Guaza, 1995). Grooming behaviors, which may be evoked in rats by placing them into novel environments (Dunn, Green, & Isaacson, 1979) or by ACTH injections (Isaacson, Hannigan, Brakkee, & Gispen, 1983), are reduced to normal levels by peripheral injections of L-NAME (Poggioli, Benelli, Arletti, Cavazzuti, & Bertolini, 1995). In the present study, we tested rats in the open field 7 d following termination of L-NAME and 0.5 Hz magnetic field treatments. Of particular interest were the statistically significant quadratic terms for the post hoc analyses of several open field variables. The results showed increased incidence of three behaviors (ambulation, rearing, grooming) for rats that been exposed to the microT, 0.5 Hz magnetic fields, or to L-NAME but "return to baseline values" for rats that had been given both treatments (i.e., similar to rats receiving neither treatment).

The findings that rats: 1) exposed to the microT, 0.5 Hz magnetic fields displayed equivalent behaviors to rats given L-NAME, and 2) given both agents displayed behaviors indistinguishable from water-treated/reference-exposed (nanoT) rats, suggest a pro-nitric oxide effect of the microT magnetic fields which counteracted the perinatal L-NAME treatment. These results would be consistent with the report of Kavaliers et al. (1998). However, the chronicity of the magnetic field effect must be longer after field-termination than the chronicity of L-NAME after suspension of drug treatment (e.g., before the pro-nitric oxide rebound) because rats in both of these groups

displayed behaviors consistent with enhanced nitric oxide activity during open field testing.

To suggest that the microT magnetic fields and L-NAME both acted upon similar pathways during their application periods would be compatible with the observation that similar behaviors were displayed by these two groups upon termination of treatment. The experimental observation that the microT magnetic fields "cancelled" the behavioral effects of L-NAME when co-administered is more difficult to explain. Moreover, this effect is not obviously congruent with the rapid return to homeostatic levels of nitric oxide-related enzymes following termination of L-NAME treatment (Prickaerts et al., 1998; Virgili et al., 1999) and the marked hyperactivity of rats following exposures to ELF complex magnetic fields. This effect persisted for at least 21 d following termination of magnetic field treatment (Persinger et al., 2001).

Long-term changes in the speed or intensity of associative learning for adult rats given the perinatal treatments were not evident. However, residual changes in baseline motor activity (as inferred by the numbers of midline chamber crossovers prior to context/shock pairings during the contextual fear conditioning paradigm) and for latencies to respond to thermal stimuli, were evident. Although motor activity and thermal analgesia are both affected by acute L-NAME administration, the vector of the effect appears to be influenced by animal strain (Seaman, Belt, Doyle, & Mathur, 1999). For instance, Moore, Oluyomi, Babbedge, Wallace, & Hart (1991) have reported that acute NOS inhibition dose-dependently (1–75 mg/kg) increased latencies in the formalin pain test while large doses of L-NAME (600 mg/kg) reduced baseline motor activity.

Seaman et al. (1999) have demonstrated that the thermal latency (to back paw licks) following 50 mg/kg L-NAME increased by approximately 100% relative to untreated mice. This same dose of L-NAME increased motor activity (assessed by light beam interruptions in an open field environment) by approximately 20% relative to untreated mice. This latter finding that L-NAME increased motor activity is quite paradoxical to the typical ambulation-reducing effects of L-NAME (Sandi et al., 1995). Although the augmentation of thermal antinociception by L-NAME treatments in the Wistar rat has been demonstrated (Dixon & Persinger, 2001), the effects of

this treatment on baseline motor activity in this strain have not. In the context of the findings reported by Seaman et al. (1999) that L-NAME increased both thermal latencies and baseline motor activity (and given that termination of chronic L-NAME and magnetic field treatment in the present study may have caused a nitric oxide rebound) the findings of chronic suppressions in motor activity and decreased thermal latencies may suggest a long-term but subtle overshoot in nitric oxide activity (e.g., a long-term upregulation of nitric oxide synthase activity). The ontogenetic enhancement of neurotransmitter activity, such as dopamine with the corpus striatum, has been reported for rats exposed prenatally to more intense (50 mT) static magnetic fields (Lee et al., 2001).

The trend toward reduced baseline motor activity was shared between perinatal microT magnetic field-exposed rats and perinatal L-NAME-treated rats, while the reduced hotplate latency was only noted for rats treated perinatally with L-NAME. This pattern may suggest that the perinatal drug and magnetic field treatments affected two different isoforms of NOS—one isoform implicated in thermal antinociception, the other isoform implicated in both ambulation and processing nociceptive stimuli. It may be relevant that pulsed DC magnetic fields only affect NOS activity when the magnetic fields are presented in combination with lipopolysaccharides (Yoshikawa et al., 2000), a finding consistent with an effect strictly on inducible nitric oxide synthase activity (iNOS). L-NAME inhibits two isoforms of NOS—endothelial nitric oxide synthase and neuronal nitric oxide synthase (eNOS and nNOS, respectively). The specific roles of eNOS and nNOS for ambulation and thermal antinociception and their regulation by specific types of magnetic fields await further investigation.

The caveat to our interpretations assumes that the strengths of the fields generated by the reference coil (1 to 13 nT) resulted in behavioral changes much weaker than those produced by the activated coils (790 to 1,770 nT). If intensity were the critical factor for inducing perinatal changes in development, then the stronger fields (which masked any perturbations from geomagnetic activity) would have been most effective. However, we cannot exclude the possibility that the nanoT level magnetic fields induced within the reference coil may have been more biologically effective. If this

alternative is valid, then our interpretations for the direction of the effects would be reversed.

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